Technology Progression In Plants Used For Food and Medicine

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Over the past 10,000 years, humans have affected crop evolution by selecting and growing plants for dietary and medicinal purposes. Creation of the crops that provide our present food supply has been a dynamic and rapid evolutionary process resulting from selection for palatability and for nutritional and health-related traits. Comparatively less effort has been devoted to improvement of therapeutic or pharmacological properties, although new technologies are making this much more feasible. The first section of this report will focus on New-World species of the *Solanaceae* (the potato/tomato/tobacco family) to illustrate selection processes we have used to create new foods, with some mention of the value of new DNA-based tools for future improvements. Secondly, I will discuss biotechnological tools now being used to convert wild plants to "cultured materials" that may provide superior pharmaceuticals such as anti-cancer agents. Lastly, the potential for using modern biotechnology is discussed as a means of creating "modern herbal medicines," *e.g.* crops as sources of oral vaccines.

CROP DOMESTICATION—REMOVING TOXICITY

Potato (*Solanum tuberosum*) and its relatives evolved in the central Andes of Peru and Bolivia. More than a hundred wild species of tuber-bearing *Solanum* can still be found in the mountains of South America. Chemical analyses show that these tubers contain many toxic chemicals, including glycoalkaloids (which give a bitter taste), saponins, phytohemagglutinins, proteinase inhibitors, sesquiterpene phytoalexins, and phenols. These chemicals provide protection against attack by fungi, bacteria, and insects, and certainly also deterred our ancestors from eating them because of with their bitter taste and toxicity. About 6,000 years ago, a strategy for converting toxic potatoes to a palatable foodstuff was developed, and is still reflected in customs of modern Andeans when they collect wild tubers and make tunta. Bitter-tasting potatoes are spread on the ground at high altitudes to freeze overnight, and then are walked upon to fragment them. Lying in the dry mountain air and going through cycles of freezing and thawing results in a "freeze-dried" product that is then placed in a depression along a running stream. The flowing water leaches out over 90% of the toxins, leaving the tunta for consumption. (One is reminded of instant mashed potatoes, albeit with much less work involved in their preparation.)

It is likely that ancestral "farmers" tasted the potatoes they collected and preserved and replanted any that were less bitter. This early domestication led to preferred "varieties" that could be grown at lower altitudes, since they no longer needed the dry, cold mountain nights for freeze drying. Several thousand varieties of potatoes are grown today in the Andean region, of varied flavor and nutritional value; they represent the trial-and-error selection of many, many farming generations. By the late sixteenth century, early explorers of South America who had developed a taste for the domesticated potato brought some varieties to Europe, whence they were later transferred to North America and other parts of the world. Today, potato is the world's fourth most important food crop (after wheat, maize, and rice); we can thank our American ancestors for the "genetic improvement" that made this possible.

Scientists interested in how foraging humans developed agricultural societies have documented other examples of crop domestication over the last 10,000 years. The introduction of new foods has also been studied, with examples of slow acceptance. For example, tomatoes, which are in the same family as potatoes and also originated in South America, have only recently been regarded as edible. In the nineteenth century, Europeans and Americans believed they were poisonous. (There was logic to this assumption; wild tomatoes contain highly toxic compounds, especially alkaloids, much like the American wild relative—black nightshade (*Solanum nigrum*)—which we still avoid due to its toxicity. Early introductions of tomato to Europe may have been for ornamental value related to pretty flowers and colorful fruit.) As recently as 1820, the state of New York forbade tomato consumption; the edict was changed when Colonel Robert Johnston announced that he would eat an entire bag of them outside the courthouse in Salem, New Jersey. It is reported that two thousand people turned up to watch him die, and a band played a funeral march while Johnston ate the lot and announced: "This luscious, scarlet apple will form the foundation of a great garden industry." He was correct; genetic improvement of tomatoes has led to a multitude of sizes, shapes, colors, and tastes (Figure 1). Some of those known today as "heritage varieties" date back to selections from the early 1900s.

Unintended Outcomes of Toxin Removal

The domestication of virtually all of the world's major food crops has involved the selection of varieties that have lost their genetic capacity to make toxic chemicals. While this is clearly of advantage for human digestion, it leaves the

Figure 1. Genetic diversity in tomatoes is evident in fruit size, color, and shape. Biochemical variability is less obvious, especially with respect to secondary metabolites such as toxic alkaloids.

plants with a greatly reduced defensive capacity against pathogens and predators (fungi, bacteria, and insects). (Toxic chemicals are nature's pesticides; weeds, which often have a bitter taste due to the presence of these chemicals, generally resist disease and predation better than crops.) About 300 years after introduction into Europe, potatoes wre attacked by late-blight disease (caused by the oomycete *Phytophthora infestans*) in the devastating Irish potato famine of 1845 and 1846. *Phytophthora* had probably been a pathogen on other species, but mutations allowed it to alter its host range to include potato, especially "chemically weakened" domesticated varieties.

Agricultural specialists have developed alternative chemical strategies to improve crop defenses against pathogens and predators. In the nineteenth century, various "pesticide" formulations were developed in attempts to protect potatoes, grapes, and other crops. These included sprays containing copper or arsenic, or nicotine in tobacco juice. Nicotine, a toxic alkaloid, is similar in chemistry to compounds found in other members of the *Solanaceae*, including some in tomato and potato that have been "genetically reduced" during domestication. (It is ironic that we genetically removed "defense" molecules from some crops, and then sprayed them with analogous compounds to limit disease and insect predation!) In the twentieth century, improvements in chemical synthesis allowed the development of many new classes of pesticides. In spite of very sizable expenditures by farmers who continue to purchase pesticides to fight diseases and insects, pests are still the primary cause of yield losses. We now know that many commercial pesticides mimic, at least partially, the actions of the defense chemicals that were originally in our food crops, but have been lost during domestication. In recent decades, many agricultural scientists have pursued the selective genetic restoration of "defense chemicals" to our crops, but without their inclusion in harvested portions that are eaten. This is a complex process, but one that is greatly aided by modern genomics research that defines genes and genetic elements that regulate metabolic pathways. It can be anticipated that the combined tools of DNA-based, marker-assisted breeding and gene transfer will foster guided evolution for further improvements in crop quality and resistance to pests and pathogens.

The power of new techniques is already apparent in the results of efforts of crop breeders to modify domestic potatoes: genetically modified varieties are now commercially available. One of the first targets was insect resistance, since predation (especially by the Colorado potato beetle) is among the most important reasons for yield losses, and prevention sometimes requires several applications of insecticide. In addition, loss of harvested tubers to insect larvae is a significant problem in developing countries lacking adequate storage facilities. The strategy first used to create insect-resistant potatoes involved transfer of a gene from a bacterium that is pathogenic to insect larvae, but which is harmless to birds, fish, and mammals. The bacterium, *Bacillus thuringiensis*, produces an insecticidal protein in nature (the *Bt* protein). When an insect larva eats plant tissue containing the *Bt* protein, its digestive process is fatally interrupted. This approach offers reduction in crop losses and, in parallel, less use of chemical insecticides.

Selection of Plants for "Valuable Toxicity"

In addition to developing food crops with reduced toxin content, our ancestors used a trial-and-error approach to identify plants for treatment or prevention of disease. Herbal extracts have been used for thousands of years and still comprise the primary medicinals used by nearly two thirds of the world's population. About 30% of "western" medicines utilize plant products in their formulation or synthesis. Although our ancestors could not have described it in modern terms, we now know that they were selectively identifying plants or plant parts that contain complex chemicals that can directly modulate human metabolism. With the advent of modern chemical analyses coupled with the emerging tools of DNA-based genomic characterization of plants, the search for new bioactive molecules is progressing rapidly.

One of the goals of cancer chemotherapy and prevention is the discovery of compounds that are relatively selective of tumor cells and, therefore, have little effect on healthy tissue. By extracting chemicals from many plant species and analyzing the mixtures for activity in cancer-cell assays, we discovered that certain triterpene saponins (called avicins) from the desert tree *Acacia victoriae* are selectively toxic to tumor cells at very low doses (Joshi *et al.*, in press). To extend this research to human clinical studies we developed a transformed "hairy-root" culture system as a reliable means of production of avicins (Figure 2). Culture conditions have been optimized for root biomass production, and we have identified putative triterpene "metabolic clusters" with enhanced activity against tumor cells. This system provides sufficient material for clinical trials, and also a means of correlating structure of individual triterpene glycosides with specific target activity in mammalian cells.

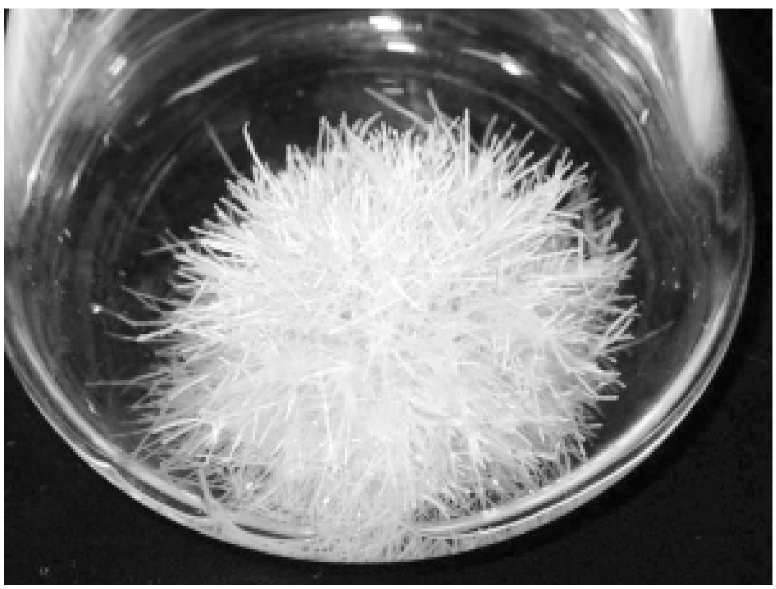

Figure 2. A "hairy root" culture established by genetic transformation of *Acacia victoriae* using *Agrobacterium rhizogenes* for gene transfer. These cultures have the advantage of immortal root growth in fermentation tanks, with uniform and predictable metabolic performance necessary for commercial pharmaceutical production.

To relate our studies of “avicins” to the broader picture of drug discovery, it is noteworthy that plants contain tens of thousands of complex chemicals, many of potential value as “yet to be discovered” biologically active molecules for use as pharmaceuticals or nutraceuticals. This is a very active area of discovery research in public-sector and industrial laboratories. As new prototype products are identified, the tools of agricultural biotechnology will increasingly be used to establish reliable and uniform sources of pharmaceutical supply.

Next Generation Agricultural Biotechnology Products

Over the last decade, my colleagues and I have adapted the tools of plant biotechnology to the area of vaccine technology. Our primary motivation came from the need for less expensive vaccines in the developing world and for technology to allow developing countries to rapidly expand in-country manufacture of vaccines. According to the World Health Organization, more than 5 million children in developing countries die each year from common infectious diseases, predominantly those that cause diarrhea and respiratory ailments. Although preventative medicine has progressed rapidly in the last decade as biotechnology has been applied to create new vaccines, the new products are comparatively expensive for developing countries. For this reason, a novel strategy has been developed for vaccine production using transgenic plants that contain genes derived from bacteria or viruses that are pathogenic to humans. The “transgene” causes the plant to produce a protein that is the “antigenic signature” of the disease. Using mice as a model, we have shown that consumption of transgenic plant samples as food triggered an oral immune response to the “signature” protein.

Research on plant-based vaccines has progressed to human clinical trials, three of which have been conducted in the United States. All were conducted after the Food and Drug Administration evaluated and approved the protocols. Vaccines to prevent diarrhea were chosen for two early studies since it causes approximately 2.5 million infant deaths annually, chiefly in the developing world. The human studies have now been completed—Phase I trials that verified the safety and efficacy of the approach (Figure 3).

To accomplish oral immunization of infants using transgenic food, it is necessary to select an appropriate crop that can be grown in developing countries, and which is eaten uncooked, to avoid destruction of the vaccine proteins by heat. Accordingly, efforts are underway to develop vaccine-synthesizing tomatoes and bananas. Current research is identifying ways to prepare a dry formulation of vaccine-containing tomato extract using common food processing technology, and to cause the appropriate proteins to accumulate in the banana fruit for infant vaccination with a food puree. In both cases, our objective is to develop agricultural and food-based technologies that can readily be adopted in developing countries.

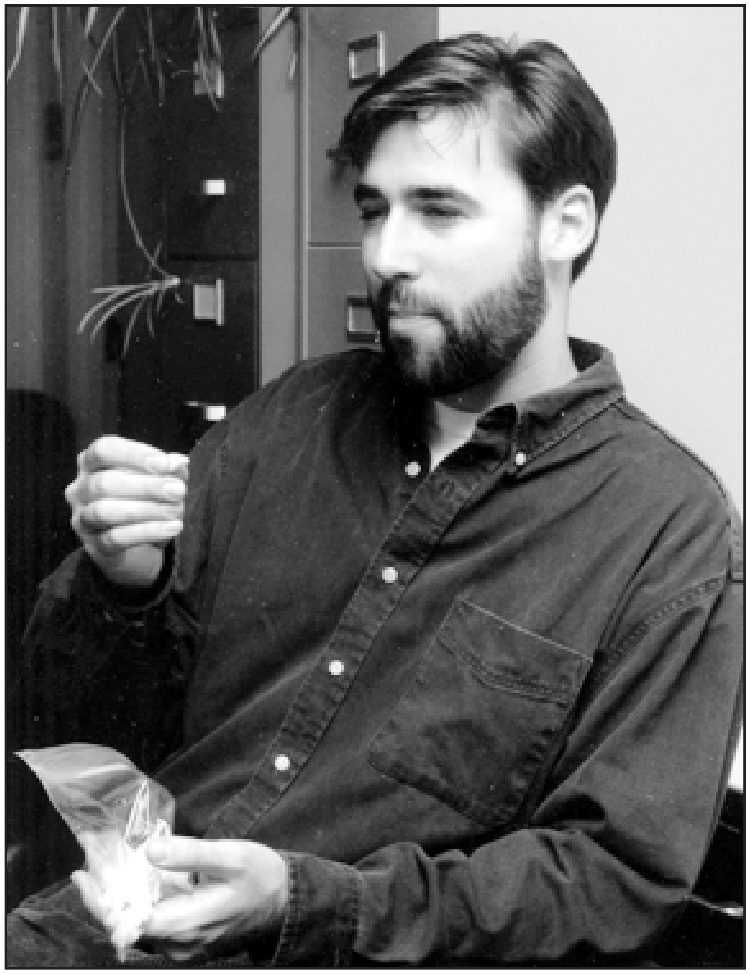

Figure 3. Human clinical trials have been conducted to test the effectiveness of transgenic potatoes as oral vaccines. This volunteer, shown eating raw potato, was part of a successful vaccine trial; plant tissues were engineered to accumulate a specific protein normally produced by a bacterium that causes severe diarrhea. When the potato samples were eaten, the immune system of the volunteers responded by production of antibodies specific to the bacterial protein, thereby providing evidence for success of a "plant-derived oral vaccine."

The use of transgenic plants to produce and deliver oral vaccines also has applicability as novel strategies for disease prevention in animals, thereby improving the safety of our food supply, and stability of animal production.

Reference and Further Reading

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